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Apparatus and Technique for the Study of the Egg Parasites of the Beet Leafhopper

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INTRODUCTION

In studying the life history and field activities of the egg parasites of the beet leafhopper (*Eutettix tenellus* (Baker)) it was necessary, because of the extremely small size of these parasites and their occurrence in the host eggs under the epidermis of the plant, to develop special apparatus and technique for handling them. Relatively few detailed field observations have been made of the seasonal occurrence of parasites attacking cicadellid eggs deposited within plant tissues, possibly because of the lack of proper equipment for such work.

METHODS OF STUDY

One of the first needs was the development of a method for adequately determining seasonal fluctuations of parasite populations in a given unit of plant material. To determine the percentage of parasitization, attempts were made to count parasitized eggs in the field or to judge the number from material brought into the laboratory, but

[1] The writer is greatly indebted to R. A. Fulton for many valuable suggestions in connection with the development of various pieces of equipment, especially the cooling device; and to A. C. Cole, Jr., for assistance in obtaining some of the field collections. Acknowledgment is made to P. N. Annand, who was in charge of the laboratory when most of the equipment and technique were developed; to C. P. Clausen, W. C. Cook, and workers of the beet leafhopper project for helpful suggestions offered during the course of the study; and to H. A. Waters for the photographs of some of the apparatus.

because of the time involved and the great difficulty in locating the eggs this method was soon discarded.

One method, in which beet petioles were soaked in a 10-percent solution of chloral hydrate for about 1 week, proved to be fairly successful, the white leafhopper eggs being easily distinguished from the plant tissue, which had turned a dark olive. The darkened parasitized eggs could also be detected easily. This and other field methods, however, gave no information as to the specific identity of the host eggs and parasites present, as several species of leafhoppers and egg parasites usually inhabit the same environment. Furthermore, an accurate parasite count could not be obtained by this method, since parasitized eggs could not be determined as such until after they had become darkened, and it is obvious that many which had not yet reached this condition would not be counted.

It appeared, therefore, that a method of counting that would allow determination of the species was necessary, and that the only practical method of obtaining these data was to rear and determine the insects contained within given units of host-plant material collected from the field.

If the parasite counts are to be of any value in determining seasonal fluctuations, all the collections must be made under conditions as nearly comparable as possible. This involves not only the method of gathering the samples in the field but also the proper handling of this material until emergence has been completed in the laboratory. The plant material must be held at a constant temperature so that any mortality occurring in the rearing cages, either before or after emergence, will be relatively uniform. Furthermore, since the separation of the parasites from the plant material depends on phototropic response, and as the physical separation of insects from debris is too tedious and expensive to be practical, it is essential that the rearings be made under constant conditions of light, thus insuring the attraction of a consistent percentage of the emerging parasites to the collecting end of the rearing cages. A series of cabinets was therefore installed in the basement of the laboratory at Twin Falls, Idaho, for the incubation and rearing of parasites and their respective host eggs under constant conditions of light and temperature.

The egg parasites[2] used in testing the various pieces of equipment as they were developed were the ones most commonly reared in southern Idaho from plant material containing beet leafhopper eggs. *Aphelinoidea plutella* Gir. and *Abbella subflava* Gir. are two trichogrammatids found widely distributed both in Russian-thistle breeding areas and in beet fields, while the two mymarids *Polynema eutettixi* Gir. and *Anagrus giraulti* Cwfd. seem to be more or less confined to beet fields.

INCUBATION AND REARING CABINETS

The set of rearing cabinets (fig. 1) has 10 compartments in all, each holding 18 cylindrical cages in a rack. By means of a thermo-

[2] Determinations were made by A. B. Gahan, of the Division of Insect Identification, Bureau of Entomology and Plant Quarantine.

stat (*a*) and relay (*b*) connected to two 500-watt cone heating units located within the inlet pipe (*c*), through which air is constantly being drawn into the cabinets by a blower (*d*), the temperature of the air within the cabinets is held at 80° F., as recorded by the thermograph (*e*). The basement air was often found to be too warm to permit the use of 80° F. until an adequate cooling system was installed in which the air from the intake (*f*) passes through a chamber (*g*) filled with a very fine spray of water from the city supply. A constant light intensity is maintained in the cabinets by a bank of electric light bulbs mounted on a sliding frame (*h*).

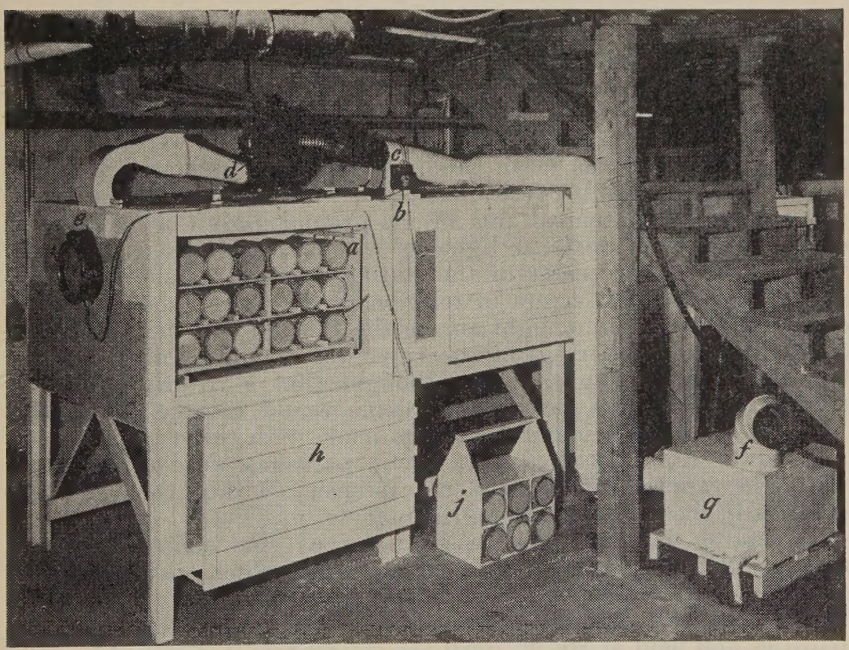

FIGURE 1.—Cabinets used for rearing the beet leafhopper egg parasites: *a*, Mercury thermostat; *b*, relay; *c*, heating units; *d*, blower fan; *e*, thermograph; *f*, air intake of cooling device; *g*, cooling chamber; *h*, sliding frame containing lights; *i*, ground glass; *j*, carrying rack.

Two thicknesses of double-strength ground glass (*i*) spaced between the lights and the rearing cages diffuse the light so that each rearing tube is subjected to about the same illumination.

EXPERIMENTS WITH LIGHT

To determine the most desirable amount of illumination for the attraction of egg parasites encountered in the area being studied, several experiments were conducted in which various light intensities were used. In these tests wattages of from 66 to 480 per cabinet were tested, giving a light intensity of from 4.8 ± 0.1 to 47.6 ± 1.8 footcandles within each rearing cage (table 1).

TABLE 1.—*Footcandles of light experimented with for the attraction of egg parasites and the number and size of lights per cabinet required to produce these intensities*

Number and description of lights	Intensity	Total wattage	Footcandles (in rearing cage)	Error divided by the mean
	Watts	*Watts*		*Percent*
11 clear	6	66	4.8±0.1	2.1
	10	110	8.3± .4	4.8
8 frosted	25	200	16.8± .8	4.8
11 frosted	25	275	24.0±1.2	5.0
8 frosted	60	480	47.6±1.8	3.8

In measuring the light intensity within the cylindrical rearing cages a footcandle meter was used, the absorbing unit being held within the cloth-covered cap in the 18 positions normally occupied by the rearing cages (fig. 1). Two sets of readings were taken for each position, and the mean candle power was calculated for each cage. When the bulbs were distributed uniformly, a higher intensity existed in the central area of the ground glass than nearer the edges. The most uniform lighting was obtained when no lights were placed in the center of the sliding frame. As shown by the size of the standard errors in relation to the mean footcandles per cage, the distribution of light among the 18 cages was quite uniform. Since the cloth covering on the caps was found to absorb about 65 percent of the light, it was thought that a covering of some transparent material such as cellophane would greatly reduce this loss. In the limited number of tests made with this type of covering, however, it appeared that fewer parasites were attracted to the light, apparently because the intensity within the cage was practically the same as that on the outside.

In testing the comparative efficiency of the various light intensities, three experiments were conducted in which nearly identical samples of dry Russian-thistle (*Salsola pestifer* A. Nels.) and sugar beet leaves were placed in the cabinets illuminated at various intensities and held at 80° F. until the emergence of insects from this material was completed (table 2).

TABLE 2.—*Comparative efficiency of various light intensities in attracting egg parasites and leafhopper nymphs to the front of rearing cages*

Test	Cages in each sample	Species	Insects attracted at each light intensity (footcandles[1])						Columns compared	Difference between means	Difference divided by error (*t*)
			47.6 (1)	24.0 (2)	16.8 (3)	8.3 (4)	4.8 (5)	Natural light[2] (6)			
	Number		*Number*	*Number*	*Number*	*Number*	*Number*	*Number*			
A	28	*Aphelinoidea plutella*	65.3±5.3	59.4± 6.5					1-2	5.9± 8.3	0.7
		Abbella subflava	3.6± .6	3.3± .8					1-2	.3± 1.0	.3
B	15	*Eutettix tenellus*		19.7± 1.5	22.7±1.2			22.0±1.8	3-2	3.0± 1.9	1.6
									6-2	2.3± 2.3	1.0
									3-6	.7± 2.2	.3
		Aceratagallia fuscoscripta		7.5± .7	9.1± .9			8.5± .7	3-2	1.6± 1.1	1.4
									6-2	1.0± 1.0	1.0
									3-6	.6± 1.1	.5
		Aphelinoidea plutella		15.3± 1.1	17.9±1.5			17.1± .9	3-2	2.6± 1.9	1.4
									6-2	1.8± 1.4	1.3
									3-6	.8± 1.7	.5
		Abbella subflava		5.7± .9	5.0± .5			6.0± .5	2-3	.7± 1.0	.7
									6-2	.3± 1.0	.3
									6-3	1.0± .7	1.4
		Polynema eutettixi		6.5± .5	6.7± .6			6.7± .9	3-2	.2± .8	.3
									6-2	.2± 1.0	.2
									6-3	.0± 1.1	.0
		Anagrus giraulti		.8± .2	1.6± .4			1.3± .4	3-2	.8± .4	2.0
									6-2	.5± .4	1.2
									3-6	.3± .6	.5
C	15	*Aphelinoidea plutella*		178.4±15.6		154.0±12.0	140.9±10.6		2-4	24.4±19.7	1.2
									2-5	37.5±18.9	2.0
									4-5	13.1±16.0	.8
		Abbella subflava		42.3± 2.8		46.5± 4.8	40.7± 4.5		4-2	4.2± 5.5	.8
									2-5	1.6± 5.3	.3
									4-5	5.8± 6.6	.9

[1] Measured in cap of rearing cage.
[2] North room near window.

In every test in which comparisons are made between results obtained with the various pieces of equipment discussed in this circular, the differences between the means and the standard error of the difference are given. If the difference between the means is twice the standard error of the difference, the variation is considered significant. As shown in table 2, when the differences between any two means for the same species and same test were examined, it was found that none of them was significant, although there were two border-line cases. In general the light intensities tested were not significantly different for the rearing of these four species of egg parasites and two leafhopper hosts. A light intensity of 8.3 footcandles seemed to be satisfactory for the rearing of *Aphelinoidea plutella* and *Abbella subflava* and is the standard intensity used when these two species are contained in the sample. Since the cabinets are used for comparing the relative densities of parasite populations rather than for indicating the total absolute population present, any satisfactory degree of illumination may be used provided all collections are treated in the same manner.

An experiment was conducted to determine the total numbers of *Aphelinoidea plutella* attracted when the lights were left on for various periods of time immediately before the collection was made. With a light intensity of 8.3 footcandles and a temperature of 80° F., 16 cylindrical cages containing dry Russian-thistle were placed in each of 3 cabinets. In the first cabinet the lights were left on continuously, in the second they were turned on for 12 hours before the daily collections were made, and in the third, for 6 hours.

In the cabinet in front of which the lights were on continuously, an average of 64 parasites per cage had come to the collecting end in 24 hours. In that where the lights had been burning for 12 hours, 55 per cage had come to the front, and 6 hours' illumination attracted 47 parasites. The maximum efficiency of a given light intensity for the collection of this species can therefore be reached only when the lights are on continuously.

Experiments With Temperature and Humidity

The temperature of the cabinets is another important consideration in obtaining comparable results. Tests were made to determine the most effective temperature for quantitative rearing of four species of egg parasites occurring in south-central Idaho. Temperatures of 80° and 85° F. were selected as being the most desirable ones obtainable within the range of the heating and cooling facilities available. In conducting these tests a total of 3,720 beet leaves containing the 4 species of parasites were distributed in 88 cylindrical rearing cages, one-half of the cages being held at 80° and the other half at 85°, the light intensity being approximately the same in both cabinets. The effect of these 2 temperatures on parasite emergence within the cabinets is shown in figure 2.

From figure 2 it is evident that these two temperatures are equally favorable for the incubation of these four species of parasites, with two possible exceptions. The mean emergence of *Anagrus girault*i in test *b* proved to be significantly higher at 80° F. Since the other two tests indicated no significant difference for this species, it is probable that the unequal result in the second test was due to some

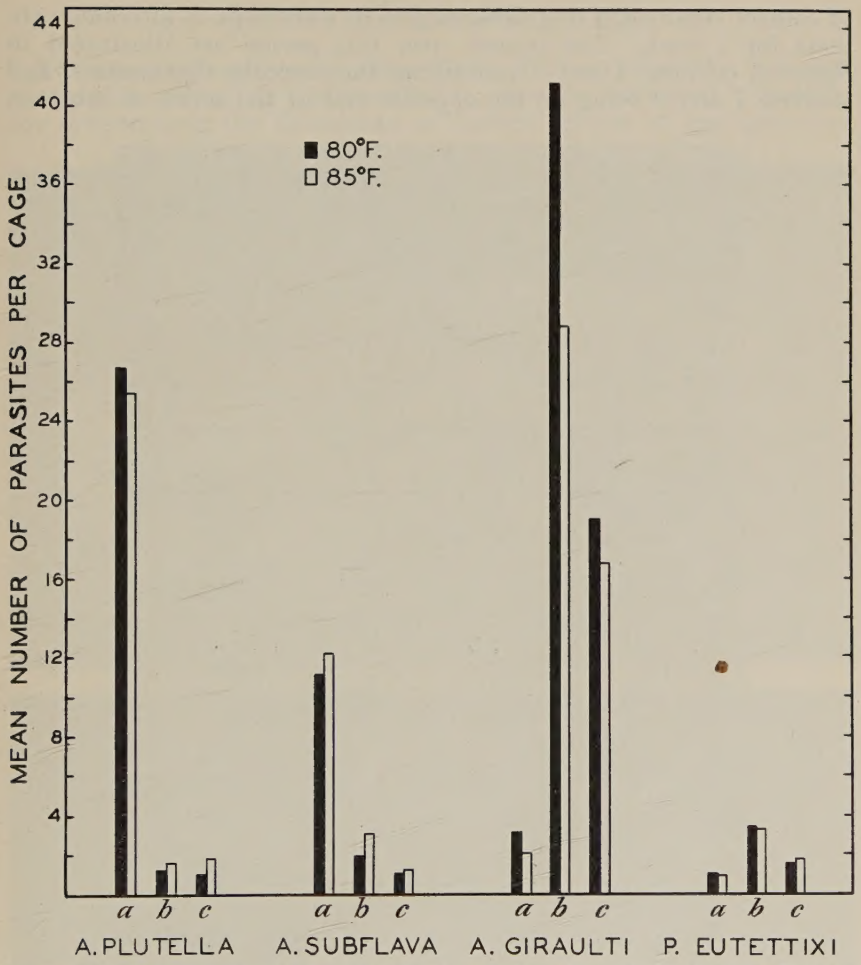

FIGURE 2.—Emergence of four species of egg parasites of the beet leafhopper at 80° and 85° F.: Letters indicate different tests.

factor other than temperature. It will be noted in figure 2, however, that smaller numbers of this species were reared at 85° than at 80° in all three tests. *A. girault*i is an extremely delicate species, morphologically, and it is thought that if 85° is less favorable for the rearing of this species, it is because of adult mortality caused by this higher temperature before the parasites are able to reach the light. In test *c* a slightly, though significantly, greater number of *Aphelinoidea plutella* were reared at the higher temperature.

Since a temperature of 80° F. proved to be as efficient as one of 85° for rearing most of the parasites studied, as well as less expensive to maintain, the cabinets are normally run at this temperature. The cabinets are in the basement of the laboratory where the room temperature does not fluctuate greatly, so this temperature is rather easily maintained, 1 blower and 2 heating units handling 6 of the cabinets containing 108 rearing cages. To demonstrate the degree

of control obtained, 4 hygrothermographs were kept in alternate cabinets for 1 week. The records over this period are illustrated in figure 3, cabinets 1 and 3 containing the mercury thermostats, and cabinets 7 and 9 being on the opposite end of the series of cabinets.

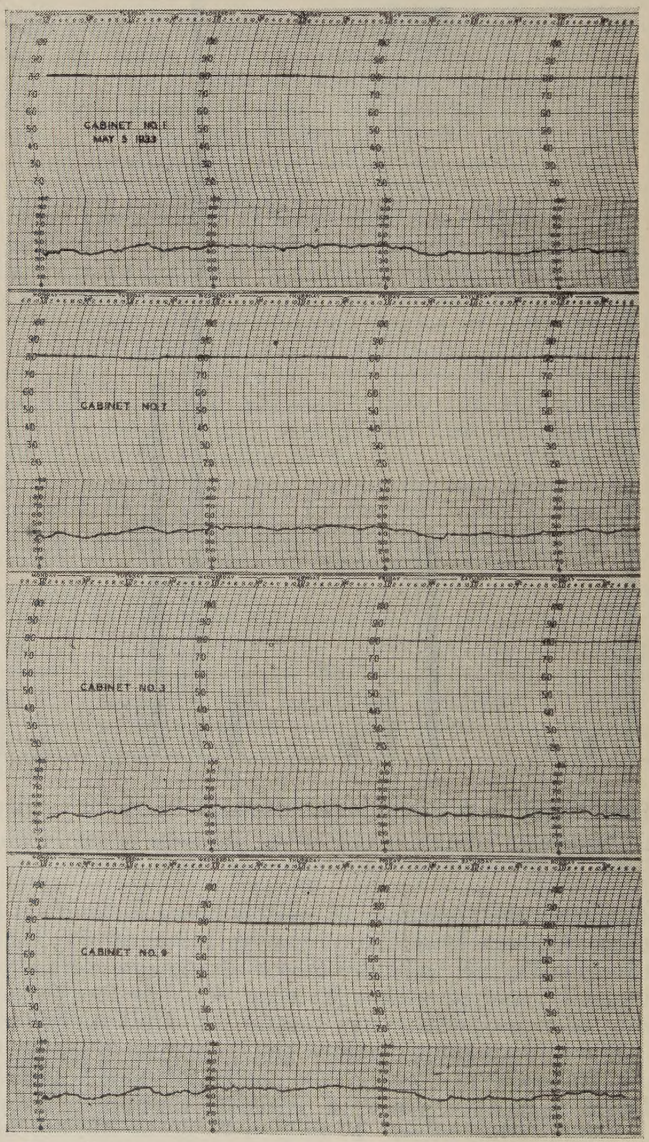

FIGURE 3.—Hygrothermograph records taken in parasite cabinets, 1 and 3 containing the mercury thermostats and cabinets 7 and 9 being on the opposite end of the series of cabinets.

While no attempt has been made to control the humidity within the cabinets, it was found that this factor ordinarily fluctuated between 35 and 50 percent relative humidity (fig. 3).

TYPES OF REARING CAGES

The usual type of rearing cage (fig. 4, *A*), consisting of a box with one or more collecting vials inserted in one end, was found inadequate for determining the abundance of certain species of egg parasites,

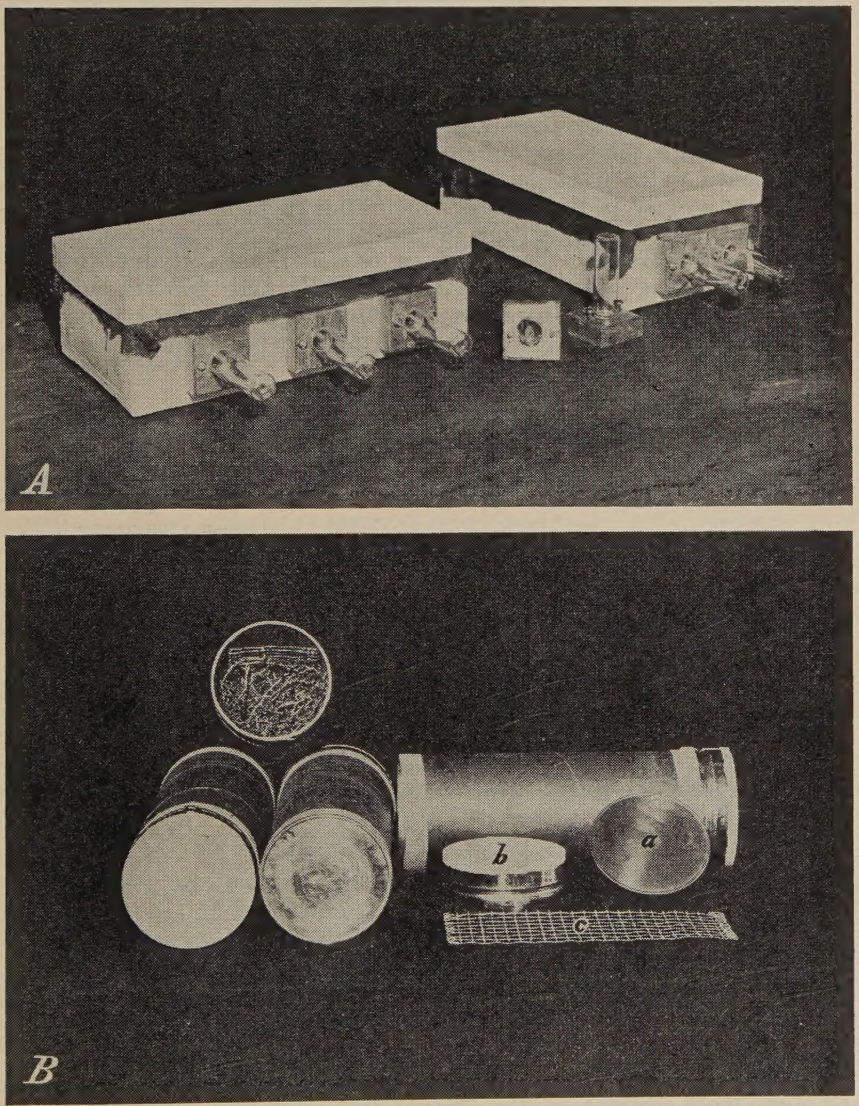

FIGURE 4.—Types of rearing cages used for studies of the parasites of the beet leafhopper: *A*, Box cages with three and two tubes; *B*, Cylindrical rearing cages: *a*, Metal cap; *b*, cloth-covered cap; *c*, 2-mesh wire-screen partition.

especially when the numbers in the sample were small. After much experimentation the rearing cage shown in figure 4, *B*, was designed, and this proved much more efficient. This cage is a cylinder con-

structed of heavy cardboard, 14 inches long and 5¼ inches in diameter, with a metal cap (*a*) covering one end and a cloth-covered cap (*b*) at the other end to be placed toward the light. A 2-mesh wire-screen partition (*c*) holds the plant material away from one side and permits a shaft of light to extend the full length of the tube, with a clear path for the parasites to the light. With this type of rearing cage very small parasite populations can be accurately determined without examination of the plant debris, since the parasites in moving toward the light concentrate in the cloth-covered cap and are retained there until collected.

Tests were conducted to determine the comparative efficiency of the box and cylindrical cages under actual working conditions. In one experiment (A) 2,880 beet leaves were divided into 3 lots, each lot being distributed evenly in the 24 cages including each of the 3 types, i. e., cylindrical cages, and box cages with 2 and 3 collecting tubes, respectively. In tests B and C the rearing cages were filled with dry Russian-thistle containing only *Aphelinoidea plutella* and *Abbella subflava*. The emergence records for four species of egg parasites from beet leaves and Russian-thistle plants contained within these three types of cages are shown in table 3.

TABLE 3.—*Emergence of four species of egg parasites of the beet leafhopper in three types of rearing cages*

Test	Cages in each sample	Species reared	Cylindrical cage (1)	Box (3 tubes) (2)	Box (2 tubes) (3)	Columns compared	Difference between means	Difference divided by error
			MEAN PARASITES PER CAGE					
	Number		*Number*	*Number*	*Number*			
A	24	*Aphelinoidea plutella*	26.7±1.5	7.6±0.6	6.4±1.4	1–2	19.1±1.6	11.9
						1–3	20.3±2.0	10.1
						2–3	1.2±1.5	.8
		Abbella subflava	11.2± .6	3.6± .5	4.1± .6	1–2	7.6± .8	9.5
						1–3	7.1± .8	8.9
						3–2	.5± .8	.6
		Anagrus giraulti	3.1± .4	2.3± .3	2.1± .3	1–2	.8± .5	1.6
						1–3	1.0± .5	2.0
						2–3	.2± .4	.5
		Polynema eutettixi	1.0± .2	.9± .2	1.2± .2	1–2	.1± .3	.3
						3–1	.2± .3	.7
						3–2	.3± .3	1.0
			MEAN PARASITES PER 100 GM.					
B	15	*Aphelinoidea plutella*	16.3±1.4	6.5±0.9		1–2	9.8±1.7	5.8
		Abbella subflava	16.4±1.2	6.1± .8		1–2	10.3±1.4	7.3
C	16	*Aphelinoidea plutella*	64.7±7.4	9.8±1.1		1–2	54.9±7.5	7.3

From table 3 it is apparent that the two mymarids *Anagrus giraulti* and *Polynema eutettixi* were recovered in about equal numbers from all cages, although the cylindrical cage appeared to be slightly preferable for rearing the former species. The cylindrical cages proved to be much more efficient for the rearing and attraction of *Aphelinoidea plutella* and *Abbella subflava*, however, probably because these two trichogrammatids move rather slowly and are thus more benefited by the increased number of chances of reaching the light offered in this type of cage. Since these two species represent the only egg parasites found in any great abundance in the principal southern Idaho breeding areas of *Eutettix tenellus*, the inadequacy of the box rearing cage for quantitative egg-parasite studies in this area is obvious.

Another test was made to determine the most desirable length of cage and amount of Russian-thistle for use in the cylindrical cage type of rearing apparatus. Dry Russian-thistle plants gathered in the field were brought to the laboratory, cut into small sections, and mixed thoroughly. This material was then divided into three lots. One lot of 80 gm. of Russian-thistle was distributed the full length of a cage; another lot, of 40 gm., was placed in the half of a cage nearest the light; and a third lot, of 40 gm., was placed in the half of another cage farthest from the light. The object was to determine whether the parasites were being attracted to the light from the farther end of the cage, or if recoveries were being made only from that portion immediately adjacent to the light. The results of this test are summarized in table 4.

TABLE 4.—*Emergence and recovery of two species of egg parasites of the beet leafhopper as affected by the position in the rearing cage and the size of the sample*

Species reared	Parasites per 100 grams of plant material			Columns compared	Difference between means	Difference divided by error
	80 gm. entire length [1] (1)	40 gm. front half [1] (2)	40 gm. rear half [1] (3)			
Aphelinoidea plutella	74.4±8.5	73.5±4.7	69.6±6.3	1-3	4.8±10.6	0.4
				1-2	.9± 9.7	.1
				2-3	3.9± 7.8	.5
Abbella subflava	8.2±2.2	8.2±1.2	9.4±2.6	3-1	1.2± 3.4	.3
				1-2	.0± 2.5	.0
				3-2	1.2± 2.9	.4

[1] Weight of Russian-thistle and position in each tube.

From table 4 it is evident that the emergence from the 80-gram sample extending the full length of the cage was as complete as that from a 40-gram sample placed either near or away from the light, and that a cage 14 inches long is just as satisfactory as one 7 inches long. Further tests made with beet leaves indicated that parasite emergence per leaf was just as great when the cage was filled as when only a portion of the space was being utilized.

TEST OF THE PERSONAL ELEMENT

The personal element involved in making collections with this type of rearing cage, using the collection box described later, was found to be practically negligible. In testing this factor daily emergence records were taken by three different collectors on three nearly identical lots of infested beet leaves. The results given in table 5 show that this factor need not be considered.

TABLE 5.—*Emergence of parasites from 50 beet leaves in each of 15 cylindrical rearing cages as determined by 3 collectors with different degrees of experience*

Experience of collector	Mean number of *Aphelinoidea plutella*	Mean number of *Abbella subflava*
	Number	*Number*
Untrained man, but with experience in the use of equipment	18.0±0.9	18.5±1.2
Trained man with experience	18.4±1.9	15.4±1.0
Trained man without experience	16.3±1.4	16.4±1.2

COLLECTING EQUIPMENT

In collecting the parasites after emergence, six cylindrical rearing cages are removed from the incubation cabinets at a time and carried into a darkened transfer room by means of the carrying rack shown in figures 1, *j*, and 5, *B*. This rack, containing the six cages filled with host-plant material from which parasites are being collected, is placed close up in front of an illuminated box (fig. 5, *A*) which contains a bank of four 25-watt light bulbs and has a double ground-glass front to diffuse the light more evenly. This is to hold the parasites in the cloth-cap end of the rearing cage while the collections are being made.

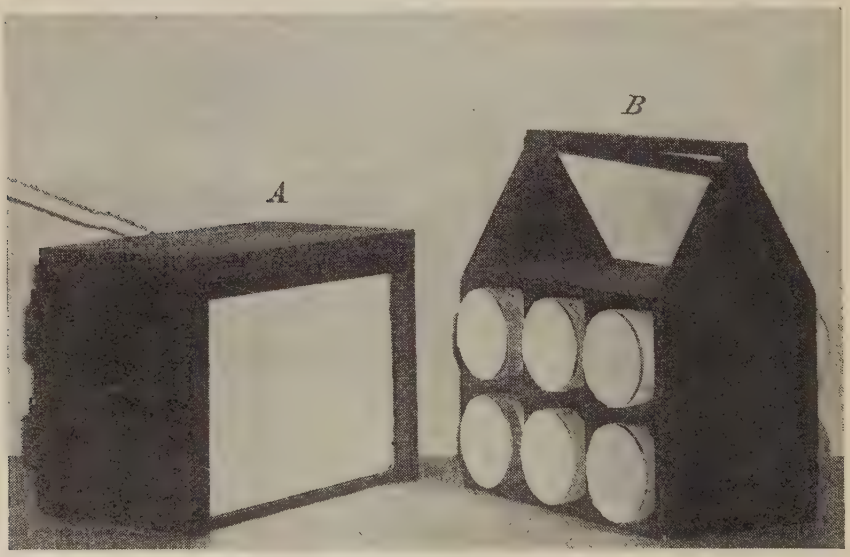

FIGURE 5.—Apparatus used in the removal of the emerged parasites that have been attracted into the cloth-cap ends of the rearing cages: *A*, Box with four 25-watt light bulbs and ground-glass front; *B*, carrying rack, showing the cloth-cap ends of the cylindrical cages. In use, this side of the carrier is placed close against the illuminated ground glass of the box to hold the parasites in these ends of the cages until each cage in turn has been withdrawn from the back of the carrier and the insects removed from the cap.

The transfer of parasites from the rearing cages into small vials partly filled with 95-percent alcohol is made through the use of the collection box. This device (fig. 6) was designed to facilitate the collection of egg parasites contained within the cloth-covered cap of the rearing cages. It consists of a box containing three 10-watt daylight-blue electric-light globes and covered with a heavy glass plate, the under surface of which is frosted. A depression 6½ inches in diameter and ⅜ of an inch deep (fig. 6, *a*) was ground out of the upper surface of the glass to hold the parasites within a small area. An electric vibrator (*b*) was mounted on the rear of the box with the arm extending just within and above the wall of the depression. A reading lens of approximately 5× magnification (*c*) was suspended on a bracket so as to be in constant focus with the insects in the depression in the glass plate.

In using this apparatus, the cloth-covered cap of the cylindrical rearing cage is held loosely against the vibrator arm while an electric contact is made by stepping on a starter switch mounted in the floor. The rapid jarring of the cap by the vibrator arm dislodges the parasites, causing them to drop into the depressed area. The insects, being positively phototropic, are held on the illuminated glass surface and may be removed by means of a wet needle or other collection apparatus designed for the purpose. It was found later that certain species were not held by the amount of light produced by the three 10-watt globes, and that many escaped before they could be collected. A 25-watt light globe was therefore installed in the box on an independent circuit, and when these species were found to occur in the sample the auxiliary globe was switched on until they had been removed.

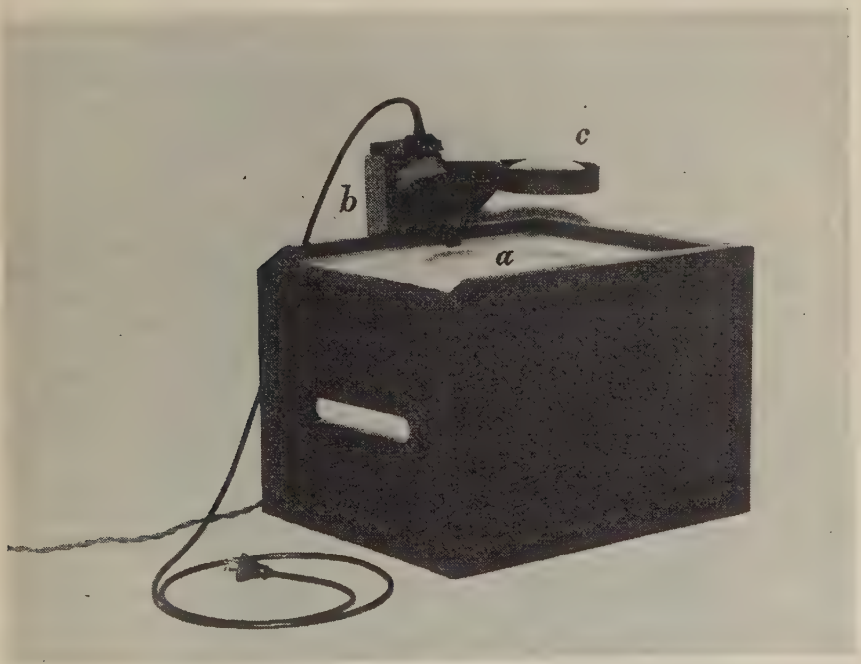

FIGURE 6.—Collecting box for egg parasites of the beet leafhopper: *a*, Depression in glass plate; *b*, electric vibrator; *c*, reading lens.

Ordinarily, a slightly moist, curved, dissecting needle was found to be the most satisfactory means of handling the parasites. This is especially true when the parasites are to be preserved immediately in alcohol. The curvature of the needle not only facilitates collecting the insects from the surface of the glass plate, but also renders the needle useful in extracting the corks from the vials. For the rapid handling of insects which are to be kept alive for further study, a vacuum apparatus using ½-dram vials was found very useful.

METHODS OF FIELD SAMPLING

In collecting samples of host-plant material containing parasites to be reared in the cabinets, the method depends largely on the type of

information sought. For example, if quantitative data are desired relative to the egg-parasite population of a given area for comparison with past or future years, the samples should be taken at random. If, as in the case of determining winter mortality and the time of spring emergence from dry Russian-thistle, the parasite population is to be expressed as the number per unit weight or volume, without regard to the area involved, the size of the sample can be materially reduced by a form of selection. This should not detract from the accuracy of the sample provided the same method is used in taking all the collections.

Weight and volume of the host plant and host-plant area are used as quantitative units in the study of egg-parasite populations at the Twin Falls, Idaho, laboratory. The volume of a plant sample is measured by means of the tank illustrated in figure 7. This tank was suggested by Carter,[3] who was also the first to suggest the use of plant volume as a unit for the study of egg-parasite populations in this project. In determining the volume of a given plant sample, the tank is first filled above the overflow tube (fig. 7, *a*) with water from the city pipes (*b*). The tank is then emptied to the overflow tube by a slight suction on the exposed end of the tube (*c*), which starts the siphon located on the upper end of this tube. When the tank has been emptied to the mouth of the overflow tube, the plant material is placed in the basket (*d*), which is then submerged. As soon as the movement of water has ceased, the water level raised by the displacement of the plant is again brought down to the mouth of the overflow tube. The displaced water is caught in a 500-cc. graduated cylinder (*e*) and the volumetric reading taken.

A series of tests was made with this volumetric device, and it seemed to be very efficient in operation. These tests were made without reference to time, the water being drawn off and the reading taken as soon as the surface of the water had become quiet. It was found later that 10 seconds was quite sufficient—a matter of importance, since there would be little absorption by the plant material in such a short time. The specific gravity of 20 samples of dry Russian-thistle, after remaining in cabinets at 80° F. for about 15 days, was found to be 0.354 ± 0.01, and the size of the standard error in relation to the mean specific gravity seems to indicate that this device under practical working conditions gives accurate results. As large numbers of samples gave practically the same results, it was concluded that the volume of dry Russian-thistle could be calculated from the weight.

Number of Units Needed in Sample

In studying the seasonal fluctuations of parasite populations with particular reference to the overwintering and spring emergence of *Aphelinoidea plutella* and *Abbella subflava*, it was desired to obtain results within a predetermined degree of accuracy. When counts are made in the field, as is possible with most insects, the degree of accuracy can be determined at the time of making the collections, any number of units being added to insure the accuracy desired.

[3] Walter Carter, formerly entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

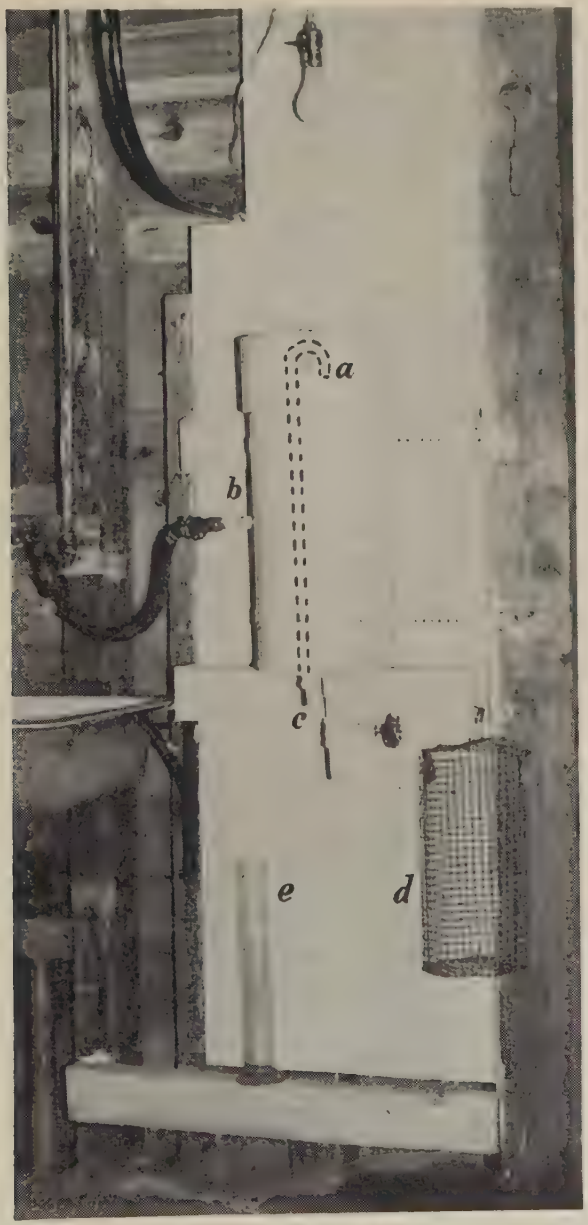

FIGURE 7.—Volumetric tank for measuring the volume of a plant sample: *a*, Overflow tube; *b*, water intake; *c*, lower end of overflow tube; *d*, basket; *e*, 500-cc. graduated cylinder.

When dealing with an insect that must be reared for a period of about 3 weeks before its numbers can be accurately determined, however, it is convenient to know beforehand the minimum number of units that must be taken as a sample. This, of course, will vary

with the mean number of parasites per cage and the uniformity of distribution per unit of host area.

In determining the spring emergence of these two parasites, large quantities of Russian-thistle from fields having fairly uniform, medium-sized plants and containing large populations of the parasites were brought to the laboratory and placed outside in as natural a position as possible under poultry-wire screen. Samples of this material were placed in the rearing cabinets at frequent intervals to determine the reduction in population due to emergence. In taking these plant samples from under the screen the random method is used, from three to five plants being placed in each cylindrical cage.

In determining the spring emergence it was thought desirable to estimate population means with a relative standard error of not over 12½ percent. Since a large number of samples of this type have been made, it was believed that by analyzing these the minimum number of cages could be determined for the past collections, and that this would serve as an index for subsequent collections taken under similar conditions. Since the first studies many more collections have been made, and the results of all are included. Relative standard errors of 12½ and 25 percent were considered, since, while the former would be preferable in determining winter mortality and spring emergence, the latter would be sufficiently accurate for many other purposes.

The formulas used were:

$$N=\left(\frac{SD}{\frac{M}{4}}\right)^2$$ for standard errors of 25 percent of the means

and

$$N=\left(\frac{SD}{\frac{M}{8}}\right)^2$$ for standard errors of 12½ percent of the means

in which N is the number of cages, SD is the standard deviation, M is the mean and $\frac{M}{4}$ and $\frac{M}{8}$ are the desired standard errors of the means.

Figure 8 summarizes all the collections of this type taken to date, giving the calculated number of cages necessary for estimating means with relative standard errors of 12½ and 25 percent. The mean number of parasites per cage was based on collections averaging the content of 18 cylindrical rearing cages. The coordinates of the open circles are the mean and the number of cages that are required in the sample to estimate the mean with a relative standard error of 12½ percent, and the closed circles show the same mean and the number of cages required in the sample to estimate the mean with a relative standard error of 25 percent. As shown by the fine broken line in figure 8, for a mean of less than 10 parasites per cage, up to about 35 cages were necessary for obtaining a relative standard error of 12½ percent, while for a mean of over 10 parasites, 15 cages would usually suffice. Eighty-six percent of all observations at the 12½ percent level fell under the fine broken line. For a standard error of 25 percent about one-fourth as many cages would be necessary. Since an analysis of all collections previously taken indicated that 15 cages would usually suffice for the accuracy desired, and it was assumed that the same variance would

occur in subsequent collections taken on the same hosts in like manner, this number was ordinarily used. Most of the records from cages having 10 parasites or less were made when emergence had practically ceased, and so for this period a larger number of cages was used.

In figure 8 the lines were drawn through the group means. These means were obtained by averaging all the individual means for each set between intervals of 10 along the x axis. Because one collection had 0.6 parasite per cage and this required 114 cages for a standard error of 12½ percent, the mean number of cages necessary for the 0–10 group is higher than would appear from the size of the individual collections. This one collection could not be plotted on a chart of this scale. The number of parasites found in this collection was abnormally low, and represented almost the completion of the spring emergence. It is realized that these figures are by no means conclusive, but they should

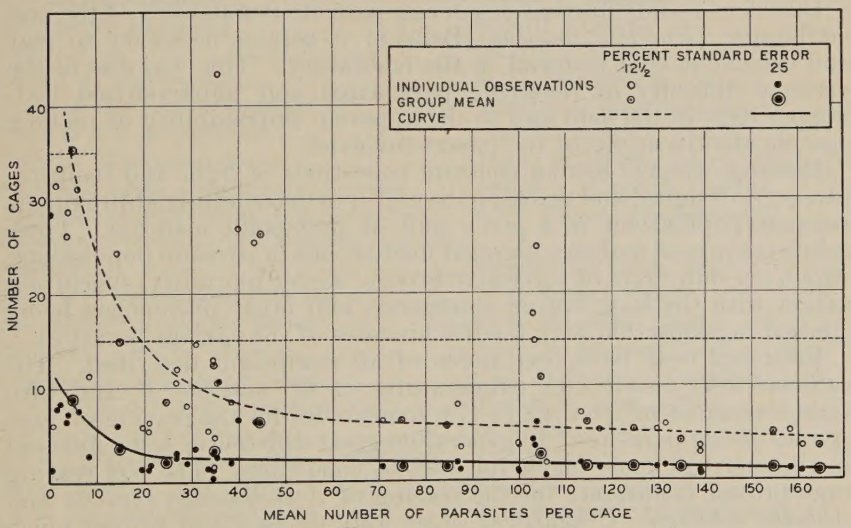

FIGURE 8.—The number of host-plant cages required to estimate mean populations of *Aphelinoidea plutella* with relative standard errors of 12½ to 25 percent.

offer a rough index as to the number of cages required. All collections were taken by the same individual, and selection of host plants was made to the extent that only medium-sized plants were brought in from the field.

THE USE OF THE EQUIPMENT AND TYPE OF DATA OBTAINED

The parasite-rearing cabinets afford a means of determining, with a fair degree of accuracy, the approximate number of egg parasites in a unit of host-plant material. This number does not express the absolute population present, since not all parasites within a given sample reach maturity, and only a certain percentage of these are attracted to the collecting end of the rearing cages. After such estimates have been arrived at under comparable conditions, however, they can be used in detecting fluctuations in field populations. These

seasonal fluctuations give indications as to successive broods, winter mortality, spring emergence, and many other phenomena that are being studied in connection with the life histories of the various egg parasites. Since the rearing cabinets are held at a constant temperature and daily emergence records are taken, estimates are obtained not only of the number of parasites present but also of the stage of development of these insects. The number of day-degrees necessary for completing development in the cabinets is indicative of that necessary in the field, and by plotting a chart showing the percentage of daily emergence of a given species at 80° F. on various dates throughout the season the number and incidence of broods represented should be indicated.

SUMMARY

In order to study fluctuations in egg-parasite populations of the beet leafhopper (*Eutettix tenellus* (Baker)) it became necessary to rear and determine field material in the laboratory. This was due to the extreme difficulty of detecting parasitized and unparasitized leafhopper eggs in the field and to the apparent impossibility of making specific identifications of the insects involved.

Rearing cabinets having constant conditions of light and temperature were designed and proved to be useful in determining approximate parasite populations in a given unit of host-plant material. These cabinets are used to detect seasonal fluctuations in parasite populations, which are indicative of successive broods, winter mortality, synchronization with the host, spring emergence, and other phenomena being studied in connection with the life histories of the various parasites.

Efficiency tests have been made of all equipment described. The cabinets were tested with temperatures of 80° and 85° F. and with light intensities of from 4.8 to 47.6 footcandles for the rearing of four species of egg parasites. In general no great differences were observed in the rearings made under the various conditions. The box rearing cage proved inadequate for the rearing of *Aphelinoidea plutella* and *Abbella subflava*. Cylindrical cages were devised and proved much more efficient for rearing these two species.

Weight and volume of the host plant and host-plant area are used as quantitative units in the study of egg parasites at the Twin Falls, Idaho, laboratory. A volumetric tank was designed and proved efficient for determining the volume of host-plant material. In sampling for egg parasites that have a rearing period of about 3 weeks before the accuracy of the sample can be determined, it was desired to know the minimum number of cages in a sample necessary for a given degree of accuracy. This minimum number has been determined in a large number of collections and should serve as an index for future collections made under similar conditions.

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